

## Cell IDx 'RightON' 100 µg Biofluor Antibody Labeling Kits

Description: Easy to use kits to incorporate optimal range of Biofluor on 90-110 µg of antibody  
 Cat. No.:

Labels	Cat#	Absorbance (nm)	Emission (nm)	Extinction Coefficient (M <sup>-1</sup> cm <sup>-1</sup> )
<b>R-PE</b>	ROP-002	498	573	1.96 x 10 <sup>6</sup>
<b>APC</b>	ROQ-002	652	657	7.0 X 10 <sup>5</sup>
<b>PerCP</b>	ROR-002	482	678	3.5 X 10 <sup>5</sup>

Application: The Cell IDx 'RightON' Biofluor antibody labeling kits have been developed to modify 90-110 µg of purified antibody @ 0.9-1.1mg/ml, incorporating the optimal range of Biofluors. Easy, efficient labeling.

- All components are included in each kit
- Available labels include: R-PE, APC, PerCP
- The only equipment needed are a pipette and a microcentrifuge
- Please ensure your antibodies for labeling are at 0.9-1.1mg/ml in buffer free of extraneous proteins such as BSA, serum or gelatin. Antibody formulations containing tris, azide, trehalose or other sugars are acceptable.

Steps:



Components:

Kit Part	Quantity	Description
<b>A</b>	1	<b>BLUE</b> -capped desalting column to buffer exchange antibody into Modification Buffer.
<b>B</b>	1	<b>GREEN</b> -capped desalting column to buffer exchange linker modified antibody into Conjugation Buffer. <b>NOTE: Store columns @4°C</b>
<b>C</b>	1	<b>RED</b> -capped desalting column to buffer exchange antibody into PBS. <b>NOTE: Store columns @4°C</b>
<b>D</b>	1	Antibody-linker reagent for modification of 90-110 µg protein at 0.9-1.1 mg/mL. <b>NOTE: IMMEDIATELY place and store linker @&lt;-20°C on receipt of kit</b>
<b>E</b>	1	Biofluor labeling reagent with linker for modification of 90-110 µg protein at 0.9-1.1 mg/mL <b>NOTE: Store dye @4°C</b>
<b>F</b>	1	Link Buffer Tube.

Storage: Antibody-Linker reagent @ -20°C, other components @ 2-8°C  
 Shelf-Life: Not determined

Protocol:

- Optimized for 90-110 µg of antibody @ 0.9-1.1 mg/mL.
- **Antibody needs to be protein carrier free, i.e., no gelatin, BSA, serum, etc.**



### Antibody Preparation

- 1) Antibody preparation- Prepare a solution of 90-110  $\mu\text{g}$  antibody at 0.9-1.1 mg/mL- Nanodrop absorbance 0.126-0.150 au ( $1.0 \text{ cm}^{-1}$  path) or plate reader.
- 2) Desalting column preparation- Break off bottom of **BLUE**-capped column and untighten the **BLUE** cap and place column(s) in collection tubes, then place in microcentrifuge and spin for 2 minutes @1500 g. Discard flow-through and collection tube.
- 3) Add antibody directly to the **BLUE**-CAPPED column, replace **BLUE** cap lightly on column, insert in a new collection tube and centrifuge for 2 minutes @1500 g.
- 4) Add flow-through from **BLUE**-capped column to Antibody Linker Reagent (Part D), pipette several times to mix, lightly vortex and incubate for 1 hour @ room temperature.

### Antibody Desalting

- 5) Desalting column preparation- Break off bottom of **GREEN**-capped column and untighten the **GREEN** cap and place column(s) in collection tubes, then place in microcentrifuge and spin for 2 minutes @1500 g. Discard flow-through and collection tube.
- 6) Add linker-modified antibody solution to **GREEN**-capped column, replace **GREEN** cap lightly on column, insert in a new collection tube and centrifuge for 2 minutes @1500 g.

### Labeling with Biofluor

- 7) Add flow-through from **GREEN**-capped column collection tube to biofluor labeling reagent tube (Part E), pipette several times to mix and lightly vortex.
- 8) Take 15  $\mu\text{L}$  of Link Buffer (Part F) and add directly to the biofluor labeling reagent tube which already contains your antibody, pipette several times to mix, lightly vortex, and incubate for 1 hour @ room temperature in the dark.

### Purification

- 9) PBS desalting column preparation - Break off bottom of **RED**-capped column and untighten the **RED** cap, place column in collection tube, then place in microcentrifuge and spin for 2 minutes @1500 g. Discard flow-through and collection tube.
- 10) Transfer labeling mixture to the **RED**-capped tube from Step 7, insert in a new collection tube and centrifuge for 2 minutes @1500 g.
- 11) You now have a biofluor conjugated antibody ready to use! The final concentration of antibody will be  $\sim 0.6$ - $0.7 \text{ mg/mL}$ . Please store final conjugate at  $2$ - $8 \text{ }^\circ\text{C}$ .

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